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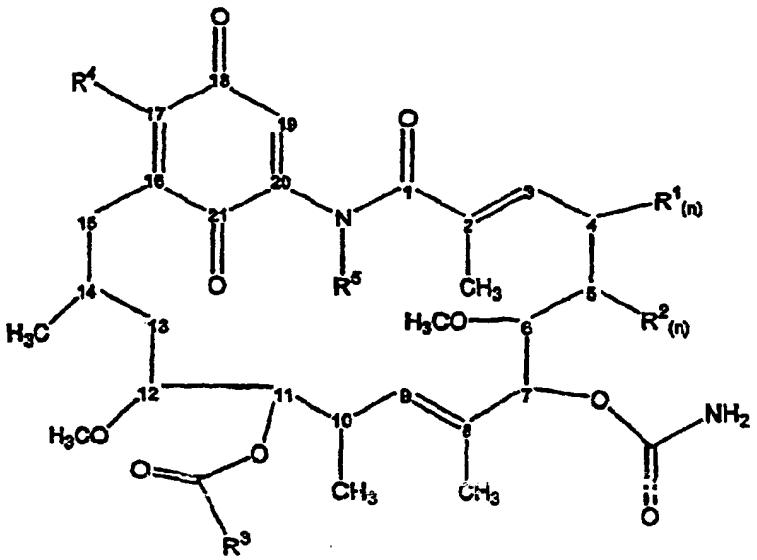
(54) Title: GELDANAMYCIN DERIVATIVES USEFUL FOR THE TREATMENT OF CANCER



(I)

(57) Abstract: A geldanamycin derivative of general formula (I) and *in vitro* and *in vivo* methods of selectively inhibiting Hsp90 in a cell without substantially inhibiting Grp94 comprising administering to a cell comprising Hsp90 and Grp94, or to a host including cells comprising Hsp90 and Grp94, respectively, the geldanamycin derivative in an amount sufficient to inhibit Hsp90 without substantially inhibiting Grp94, as well as *in vitro* and *in vivo* methods of inhibiting HIF-1α in a cell comprising administering to a cell comprising HIF-1α the geldanamycin derivative in an amount sufficient to inhibit HIF-1α, and a method of treating or preventing cancer in a host comprising administering a geldanamycin derivative to a host in an amount sufficient to treat or prevent cancer.

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GELDANAMYCIN DERIVATIVES USEFUL FOR THE TREATMENT OF CANCER

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

[0001] This patent application claims the benefit of U.S. Provisional Patent Application No. 60/246,258, filed November 6, 2000, U.S. Provisional Patent Application No. 60/279,020, filed March 27, 2001, and U.S. Provisional Patent Application No. 60/280,016, filed March 30, 2001.

FIELD OF THE INVENTION

[0002] This invention pertains to geldanamycin derivatives and methods of using the geldanamycin derivatives, for example, to treat cancer.

BACKGROUND OF THE INVENTION

[0003] The ErbB2 gene product and the HIF-1α protein are believed to play important roles in the malignancy and growth of tumor cells. Studies have shown that the over-expression of ErbB2 causes cell transformation and tumorgenesis, and *in vitro* experiments have shown that ErbB2, which is a member of the ErbB family of receptor tyrosine kinases, is required for induction of carinoma cell invasion by other members of the ErbB family. The HIF-1α protein is a transcription factor that is recognized as a pro-survival factor in many types of cancer, and some tumor cells rely on HIF-1α to stimulate many cellular processes, such as the transcriptional activation of VEGF, which is used to produce new blood vessel growth in tumor tissues.

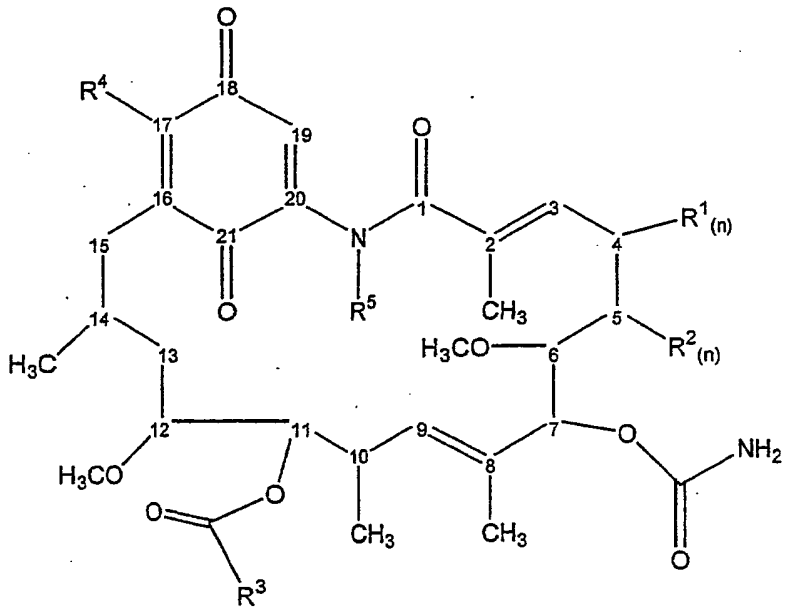
[0004] The benzoquinone ansamycin geldanamycin has anti-tumor activity *in vivo,* and has been shown to cause the rapid depletion of ErbB2 and HIF-1α protein levels. Geldanamycin has been found to destabilize HIF-1α via the proteasomal pathway and inhibits the transcriptional activity of HIF-1α (*see* Isaacs et al., *Proceedings of the AACR-NCI-EORTC Int'l Conf.*, Vol. 7, No. 11, *(Supp.)* (Nov. 2001)). Geldanamycin also is a specific inhibitor of certain chaperone proteins including the heat shock protein-90 (Hsp90) as well as the glucose regulated protein-94 (Grp94), which is localized to the endoplasmic reticulum. It is believed that geldanamycin acts on the ErbB2 protein through its inhibition of the Hsp90 chaperone protein, which is thought to be necessary to the function of the ErbB2 protein. Hsp90 also has been shown to be linked to tumor cell proliferation (see L. Whitesell et al., *Inhibition of Heat Shock Protein HSP-90-pp60v-src Heteroprotein Complex Formation by Benzoquinone Ansamycins: Essential Role for Stress Proteins in Oncogenic Transformation*, 91 Proc. Nat'l Acad. Sci. USA 8324-8328 (1994); T.W. Schulte et al., *Antibiotic Radicicol Binds to the N-terminal Domain of Hsp90 and Shares Important Biologic Activities with Geldanamycin*, 3 Cell Stress and Chaperone

Proteins 100-108 (1998); J.L. Johnson et al., *Binding of p23 and Hsp90 During Assembly with the Progesterone Receptor*, 9 Mol. Endocrinol. 670-678 (1995); W. Sullivan et al., *Nucleotides and two functional states of Hsp90*, 272 J. Biol. Chem. 8007-8012 (1997)).

[0005] Analogues of geldanamycin have been synthesized in attempts to increase the bioavailability and reduce the toxicity associated with the natural product. Among the more successful analogues is 17-allylaminogeldanamycin (17-AAG), which is currently in phase I clinical trials at the National Cancer Institute. However, there continues to be a need for other effective anticancer compounds and methods of using such compounds. The present invention seeks to fulfill such a need. The particular advantages of the invention, as well as additional inventive features, will be apparent from the description of the invention provided herein.

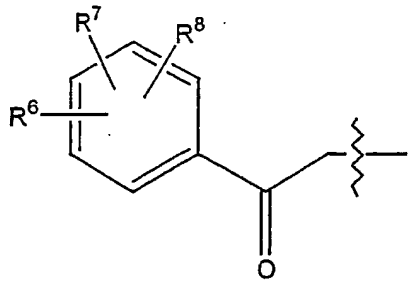
BRIEF SUMMARY OF THE INVENTION

[0006] The present invention provides a compound of formula (I),



(I)

wherein n=0 or 1 and wherein, when n=1, R^1 and R^2 are each hydrogen, and, when n=0, a double-bond exists between 4 and 5; R^3 is a C_1-C_8 aminoalkyl or a C_1-C_8 iminoalkyl; R^4 is hydrogen, a methoxy, a C_1-C_8 alkylamino, a C_1-C_8 dialkylamino, a C_1-C_8 N,N'-dialkylaminoalkylamino, or an allylamino; and R^5 is hydrogen or a group of the formula (II)



(II)

wherein each of R^6, R^7, and R^8 is independently selected from the group consisting of hydrogen, a halo, an azido, a nitro, a C_1-C_8 alkyl, a C_1-C_8 alkoxy, an aryl, a cyano, and an $NR^{10}R^{11}R^{12}$, wherein each of R^{10}, R^{11}, and R^{12} is independently selected from the group consisting of hydrogen and a C_1-C_3 alkyl; and salts thereof and prodrugs thereof.

[0007] The present invention also provides a pharmaceutical composition comprising a compound of the present invention and a pharmaceutically acceptable carrier.

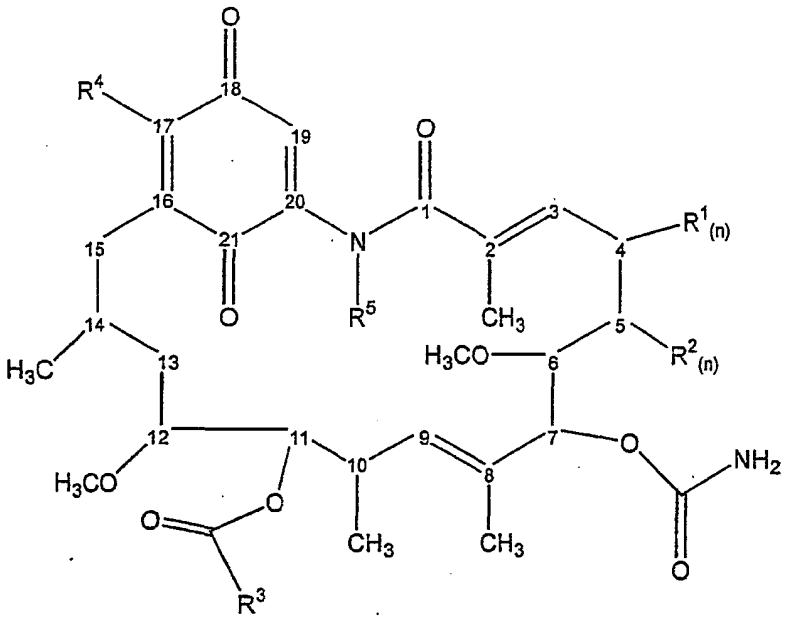
[0008] A method of selectively inhibiting Hsp90 without substantially inhibiting Grp94 in a cell is also provided. The method comprises administering to a cell comprising Hsp90 and Grp94 an above-described compound in an amount sufficient to inhibit Hsp90 without substantially inhibiting Grp94, whereupon Hsp90 is selectively inhibited.

[0009] The present invention additionally provides a method of inhibiting HIF-1α in a cell. The method comprises administering to a cell comprising HIF-1α an above-described compound in an amount sufficient to inhibit HIF-1α.

[0010] Still further provided is a method of treating or preventing cancer in a host. The method comprises administering an above-described compound to a host in an amount sufficient to treat or prevent cancer in the host, whereupon the cancer in the host is treated or prevented.

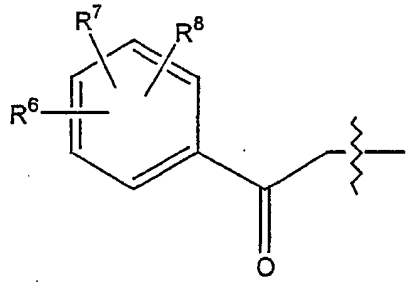
DETAILED DESCRIPTION OF THE INVENTION

[0011] The present invention provides a compound of formula (I),



(I)

wherein n=0 or 1. When n=1, each of R^1 and R^2 is hydrogen, and, when n=0, a double-bond exists between 4 and 5. R^3 is a C_1-C_8 aminoalkyl or a C_1-C_8 iminoalkyl, either of which can be substituted with one or more C_1-C_4 alkyl groups, which can be the same or different. R^4 is hydrogen, a methoxy, a C_1-C_8 alkylamino, a C_1-C_8 dialkylamino, a C_1-C_8 N,N'-dialkylaminoalkylamino, or an allylamino. When R^4 is a methoxy, a C_1-C_8 alkylamino, a C_1-C_8 dialkylamino, a C_1-C_8 N,N'-dialkylaminoalkylamino, or an allylamino, R^4 can be substituted with one or more substituents, which can be the same or different, selected from the group consisting of a C_1-C_4 alkyl, a C_1-C_8 aryl, a halo, a cyano, a nitro, an azido, an amido, and an amino. R^5 is hydrogen or a group of the formula (II)



(II)

wherein each of R^6, R^7, and R^8 is independently selected from the group consisting of hydrogen, a halo, an azido, a nitro, a C_1-C_8 alkyl, a C_1-C_8 alkoxy, an aryl, a cyano, and an $NR^{10}R^{11}R^{12}$, wherein each of R^{10}, R^{11}, and R^{12} is independently selected from the group consisting of hydrogen and a C_1-C_3 alkyl; and salts thereof and prodrugs thereof.

Positions indicated by the numbers 1-21 on the benzoquinone ansamycin ring in formula (I) represent carbon atoms.

[0012] In preferred compounds, R^3 is selected from the group consisting of an aminomethyl, an aminoethyl, an aminopropyl, an aminobutyl, a C_1-C_8 N-methylaminoalkyl, and a C_1-C_8 N,N'-dimethylaminoalkyl. Particularly preferred are compounds wherein R^3 is aminomethyl, aminopropyl, N,N'-dimethylaminoethyl or N,N'-dimethylaminopropyl. In addition, preferably n=0 and a double bond exists between 4 and 5. Therefore, particularly preferred compounds of the present invention include, geldanamycinglycinate, 17-demethoxy-17-allylamino geldanamycin-11-aminoacetate, 17-demethoxy-17-allylamino geldanamycin-11-[3-(dimethylamino)-propionate], 11-(4-aminobutyrate)-geldanamycin, and 17-demethoxy-17-allylamino geldanamycin-11-[4-(dimethylamino)-butyrate], and, especially, salts (e.g., hydrochloride salts) thereof.

[0013] Compounds of the present invention can be in the form of a salt, which is preferably a pharmaceutically acceptable salt. Suitable pharmaceutically acceptable acid addition salts include those derived from mineral acids, such as hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric, and sulphuric acids, and organic acids, such as tartaric, acetic, citric, malic, lactic, fumaric, benzoic, glycolic, gluconic, succinic, and arylsulphonic acids, for example *p*-toluenesulphonic acid.

[0014] Compounds of the present invention can be also be in the form of a prodrug. By "prodrug" is meant a compound of the present invention, wherein the R^3, R^4 and/or R^5 groups can be cleaved-off by normal metabolic processes *in vivo*, thereby transforming the prodrug back into the parent geldanamycin compound. It will be understood that prodrugs are typically used when the active drug may be too toxic to administer systemically, the active drug is absorbed poorly by the digestive tract, or the body breaks down the active drug before it reaches its target.

[0015] Preferably, the compounds of the present invention have improved water-solubility as compared to Geldanamycin and/or have the ability to form salts (*e.g.*, acid salts) having improved water solubility as compared to geldanamycin, while retaining *in vitro* or *in vivo* anticancer or antitumor activity. For example, preferred compounds of the present invention have water-solubilities of at least about 0.5 mg/ml, preferably at least about 1 mg/ml (e.g., 1.5 mg/ml), more preferably at least about 2 mg/ml (*e.g.*, at least about 2.5 mg/ml). Most preferably, the compounds of the present invention have water-solubilities of at least about 3 mg/ml (*e.g.*, at least about 3.5 mg/ml) or even at least about 4 mg/ml (*e.g.*, at least about 4.5 mg/ml) or even greater water-solubilities (*e.g.*, at least about 5 mg/ml). Water-solubilities can be determined using standard techniques known in the art. While the compounds of the present invention, preferably, have improved water-solubility, techniques known in the art can be used in conjunction with the present

invention to further improve the water-solubility of these compounds. For example, the pH of a formulation comprising the compound can be adjusted to optimize the water-solubility of the compound. Also, various additives can be incorporated into a formulation comprising a compound of the present invention. Useful additives include cosolvents (e.g., polyethylene glycol), surface active agents, complexing agents, emulsifying agents, amphiphilic compounds, liposomes, and micro- and nanoparticles, which may improve the solubility of a compound of the present invention.

[0016] Compounds of the present invention preferably have a strong binding affinity for Hsp90. As mentioned, GA is known to have a strong affinity for Hsp90 and effectively inhibits the protein's function. Preferred compounds of the present invention have a binding affinity for Hsp90 that is at least about $1/10^{th}$ or more, preferably at least about $1/5^{th}$ or more, of the binding affinity exhibited by GA for Hsp90. The most preferred compositions have a binding affinity for Hsp90 that is about equal to, or even greater than, that of GA.

[0017] Also, the compounds of the present invention preferably exhibit a binding affinity for Grp94 that is several-fold less than that exhibited by GA. GA has a binding affinity for Grp94 that is about equal to its binding affinity for Hsp90. Preferred compounds of the present invention exhibit a binding affinity for Grp94 that is about $1/20^{th}$ or less, preferably about $1/40^{th}$ or less, more preferably about $1/60^{th}$ or less, or even about $1/80^{th}$ or less, of the binding affinity for Grp94 exhibited by GA.

[0018] Preferred compounds of the present invention have a binding affinity for Hsp90 that is substantially greater than their binding affinity for Grp94. For example, the preferred compounds of the present invention have a binding affinity for Hsp90 that is at least 10-times greater, more preferably at least 40-times greater, even more preferably at least 80-times greater, than the binding affinity of the compound for Grp94.

[0019] Binding affinities and relative binding affinities can be measured by any suitable method known in the art including by copy of illustration, through the use of competitive binding assays and comparison of the concentration at which test compounds (e.g., GA or a compound of the present invention) binds to a given percentage of the target protein (e.g., Hsp90 or Grp94) in the presence of an inhibitor (e.g., another small molecule known to share, with the test compound, the same binding site on the target protein) and the like. A preferred method of determining the relative binding affinities is by comparing the concentration of the test compound at which 50% of the target protein is bound (otherwise know as the IC_{50} concentration level) in a competitive binding assay.

[0020] Without regard to the binding affinity for Hsp90, as described above, the compounds of the present invention, preferably, inhibit HIF-1α. Preferred compounds of the present invention inhibit HIF-1α to a degree that is at least about 10% or more,

preferably at least about 25% or more, such as at least about 50% or more, or even about 75% or more (*e.g*, approximately equal to or greater than) of the level of inhibition of HIF-1α exhibited by GA. The level of inhibition can be determined, for example, by measuring the level of HIF-1α activity in a cell in the presence of a compound of the present invention or in the presence of GA as compared to the level of activity in the absence of such compounds.

[0021] The present invention further provides a pharmaceutical composition or formulation comprising a compound of the present invention and a pharmaceutically acceptable carrier. The pharmaceutical composition or formulation preferably comprises a compound of the present invention in an amount sufficient to inhibit Hsp90 without substantially inhibiting Grp94 when administered to a host having cells comprising Hsp90 and Grp94. Preferably, the amount is sufficient to reduce substantially or eliminate the expression of ErbB2 proteins when administered to a host having cells that express the ErbB2 protein in the absence of the compound of the present invention. Additionally or alternatively, the pharmaceutical composition or formulation preferably comprises a compound of the present invention in an amount sufficient to inhibit HIF-1α.

[0022] The pharmaceutical composition can comprise more than one active ingredient, such as more than one compound of the present invention, or a compound of the present invention in combination with another pharmaceutically active agent or drug.

[0023] The carrier can be any suitable carrier. Preferably, the carrier is a pharmaceutically acceptable carrier. With respect to pharmaceutical compositions, the carrier can be any of those conventionally used and is limited only by chemico-physical considerations, such as solubility and lack of reactivity with the active compound(s), and by the route of administration. It will be appreciated by one of skill in the art that, in addition to the following described pharmaceutical composition, the compounds of the present inventive methods can be formulated as inclusion complexes, such as cyclodextrin inclusion complexes, or liposomes.

[0024] The pharmaceutically acceptable carriers described herein, for example, vehicles, adjuvants, excipients, and diluents, are well-known to those skilled in the art and are readily available to the public. It is preferred that the pharmaceutically acceptable carrier be one which is chemically inert to the active compound(s) and one which has no detrimental side effects or toxicity under the conditions of use.

[0025] The choice of carrier will be determined in part by the particular compound, as well as by the particular method used to administer the composition. Accordingly, there are a variety of suitable formulations of the pharmaceutical composition of the present invention. The following formulations for oral, aerosol, parenteral, subcutaneous,

intravenous, intramuscular, interperitoneal, rectal, and vaginal administration are exemplary and are in no way limiting.

[0026] Injectable formulations are among those formulations that are preferred in accordance with the present invention. The requirements for effective pharmaceutical carriers for injectable compositions are well-known to those of ordinary skill in the art (see, e.g., *Pharmaceutics and Pharmacy Practice*, J.B. Lippincott Company, Philadelphia, PA, Banker and Chalmers, eds., pages 238-250 (1982), and *ASHP Handbook on Injectable Drugs*, Toissel, 4th ed., pages 622-630 (1986)). It is preferred that such injectable compositions be administered intravenously or, in the context of the treatment of cancer, intratumorally (within the tumor) or peritumorally (near the outside of the tumor).

[0027] Topical formulations are well-known to those of skill in the art. Such formulations are particularly suitable in the context of the present invention for application to the skin.

[0028] Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the compound dissolved in diluents, such as water, saline, or orange juice; (b) capsules, sachets, tablets, lozenges, and troches, each containing a predetermined amount of the active ingredient, as solids or granules; (c) powders; (d) suspensions in an appropriate liquid; and (e) suitable emulsions. Liquid formulations may include diluents, such as water and alcohols, for example, ethanol, benzyl alcohol, and the polyethylene alcohols, either with or without the addition of a pharmaceutically acceptable surfactant. Capsule forms can be of the ordinary hard- or soft-shelled gelatin type containing, for example, surfactants, lubricants, and inert fillers, such as lactose, sucrose, calcium phosphate, and corn starch. Tablet forms can include one or more of lactose, sucrose, mannitol, corn starch, potato starch, alginic acid, microcrystalline cellulose, acacia, gelatin, guar gum, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, calcium stearate, zinc stearate, stearic acid, and other excipients, colorants, diluents, buffering agents, disintegrating agents, moistening agents, preservatives, flavoring agents, and pharmacologically compatible excipients. Lozenge forms can comprise the active ingredient in a flavor, usually sucrose and acacia or tragacanth, as well as pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin, or sucrose and acacia, emulsions, gels, and the like containing, in addition to the active ingredient, such excipients as are known in the art.

[0029] The present inventive compound, alone or in combination with other suitable components, can be made into aerosol formulations to be administered via inhalation. These aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like. They also may be formulated as

pharmaceuticals for non-pressured preparations, such as in a nebulizer or an atomizer. Such spray formulations also may be used to spray mucosa.

[0030] Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The present inventive compound can be administered in a physiologically acceptable diluent in a pharmaceutical carrier, such as a sterile liquid or mixture of liquids, including water, saline, aqueous dextrose and related sugar solutions, an alcohol, such as ethanol, isopropanol, or hexadecyl alcohol, glycols, such as propylene glycol or polyethylene glycol, dimethylsulfoxide, glycerol ketals, such as 2,2-dimethyl-1,3-dioxolane-4-methanol, ethers, such as poly(ethyleneglycol) 400, an oil, a fatty acid, a fatty acid ester or glyceride, or an acetylated fatty acid glyceride with or without the addition of a pharmaceutically acceptable surfactant, such as a soap or a detergent, suspending agent, such as pectin, carbomers, methylcellulose, hydroxypropylmethylcellulose, or carboxymethylcellulose, or emulsifying agents and other pharmaceutical adjuvants.

[0031] Oils, which can be used in parenteral formulations include petroleum, animal, vegetable, or synthetic oils. Specific examples of oils include peanut, soybean, sesame, cottonseed, corn, olive, petrolatum, and mineral. Suitable fatty acids for use in parenteral formulations include oleic acid, stearic acid, and isostearic acid. Ethyl oleate and isopropyl myristate are examples of suitable fatty acid esters.

[0032] Suitable soaps for use in parenteral formulations include fatty alkali metal, ammonium, and triethanolamine salts, and suitable detergents include (a) cationic detergents such as, for example, dimethyl dialkyl ammonium halides, and alkyl pyridinium halides, (b) anionic detergents such as, for example, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates, (c) nonionic detergents such as, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylenepolypropylene copolymers, (d) amphoteric detergents such as, for example, alkyl-b-aminopropionates, and 2-alkyl-imidazoline quaternary ammonium salts, and (e) mixtures thereof.

[0033] The parenteral formulations will typically contain from about 0.5% to about 25% by weight of the active ingredient in solution. Preservatives and buffers may be used. In order to minimize or eliminate irritation at the site of injection, such compositions may contain one or more nonionic surfactants having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactant in such formulations will typically range from about 5% to about 15% by weight. Suitable surfactants include polyethylene

sorbitan fatty acid esters, such as sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol. The parenteral formulations can be presented in unit-dose or multi-dose sealed containers, such as ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.

[0034] Additionally, the present inventive compounds, or compositions comprising such compounds, can be made into suppositories by mixing with a variety of bases, such as emulsifying bases or water-soluble bases. Formulations suitable for vaginal administration can be presented as pessaries, tampons, creams, gels, pastes, foams, or spray formulas containing, in addition to the active ingredient, such carriers as are known in the art to be appropriate.

[0035] The compounds of the present invention can be used for any purpose. As mentioned, the compounds of the present invention have an affinity for Hsp90, and can be used to inhibit Hsp90, for example, in a cell such as a mammalian cell or a human cell. Such method can be used, for instance, *in vitro* for research or diagnostic purposes, or *in vivo*, wherein the cell is in a host such as a mammal or a human, for the diagnosis, prevention, or treatment of a disease, such as cancer. *In vivo* activity can be measured by any suitable method known in the art. Suitable methods include, for example, a hollow fiber assay (HFA), xenograft testing and the like, as well as combinations of such testing. A preferred method of determining *in vivo* activity is a hollow fiber assay, as described in M.G. Hollingshead et al., *In Vivo Cultivation of Tumor Cells in Hollow Fibers, (57)2* Life Sci. 131-141 (1995). Typically, a hollow fiber assay is used as a preliminary screen to identify compounds having moderate to prominent anti-cancer activity. Generally, compounds which exhibit significant positive results are considered to have moderate to prominent anti-cancer activity. HFA can be used in conjunction with other testing methods, such as xenograft testing, to provide more evidence as to the *in vivo* anti-cancer activity of compounds and, thus, their suitability for treatment of a particular disease.

[0036] As previously mentioned, geldanamycin and derivatives thereof known prior to the present invention have approximately an equal affinity for both Hsp90 and Grp94 (*i.e.*, they inhibit Hsp90 and Grp94 with about the same effectiveness). Thus, there was previously no known method of inhibiting Hsp90 without substantially inhibiting Grp94 using geldanamycin or a derivative thereof. However, the compounds of the present invention desirably have a selective affinity for Hsp90 over Grp94, and can be used to selectively inhibit Hsp90.

[0037] Further provided by the present invention is a method of selectively inhibiting Hsp90 without substantially inhibiting Grp94 in a cell. The method comprises administering to a cell comprising Hsp90 and Grp94 a compound of the present invention in an amount sufficient to inhibit Hsp90 without substantially inhibiting Grp94, whereupon Hsp90 is selectively inhibited. The method can be used with any type of cell, such as a mammalian cell, preferably, a human cell. A compound of the present invention can be administered to such cell *in vitro*, or *in vivo,* wherein the cell is in a host such as a mammal, preferably, a human. Methods of administering a compound of the present invention to a cell *in vitro* or *in vivo* are known in the art, and include by way of example those previously described.

[0038] Preferably, the present method of selectively inhibiting Hsp90 does not inhibit Grp94 at all or inhibits Grp94 in a *de minimus* amount. However, the present method can be used to inhibit Hsp90 without substantially inhibiting Grp94, wherein the Grp94 activity level is about 50% or more, preferably about 60% or more, about 70% or more, about 80% or more, about 90% or more or even about 95% or more (*e.g.,* 97% or more), as compared to the Grp94 activity level in the absence of a compound of the present invention, or any inhibitor of the Grp94 activity level.

[0039] A compound of the present invention, desirably, is administered to a cell that expresses ErbB2 in the absence of the compound of the present invention, or to a host including such cells, in an amount sufficient to reduce or eliminate the expression of ErbB2 protein. The expression of ErbB2 is preferably reduced by at least about 20% , by at least about 30%, such as at least about 40% (e.g., at least about 50%), by at least about 70%, (e.g., at least about 80%), or even by at least about 90%, or completely, as compared to expression of ErbB2 in the absence of the compound of the present invention.

[0040] Methods by which the inhibition of Hsp90 and Grp94, or the expression of ErbB2, can be determined are generally known in the art. Inhibition of Hsp90 or Grp94 typically is determined using competitive binding assays. Exemplary methods are discussed in the Examples provided herein.

[0041] The compounds of the present invention also can be used to inhibit HIF-1α. As previously mentioned, HIF-1α is a transcription factor that is recognized as a pro-survival factor in many types of cancer, and some tumor cells rely on HIF-1α to stimulate many cellular processes, such as the transcriptional activation of erythropoietin, inducible nitric oxide synthase, and vascular endothelial growth factor (VEGF), which is used to produce new blood vessel growth in tumor tissues. Under normoxic conditions, HIF-1α is rapidly degraded by a ubiquitin ligase complex, which binds to the HIF-1α protein via a protein known as VHL. Under some circumstances, however, such as under hypoxic conditions, or in circumstances where VHL is absent from the cell or is mutated such that lacks

normal activity towards the HIF-1α protein (*e.g.*, cannot bind to the HIF-1α protein), the HIF-1α protein accumulates in the cell. Lack of VHL activity often exists, for example, in the context of a metastatic disease (*e.g.*, metastatic cancer), and is often prevalent in renal cell carcinomas.

[0042] The compounds of the present invention can be used to reduce the level of HIF-1α in a cell, thereby inhibiting the activity of HIF-1α, by destabilizing the protein and/or degrading or stimulating the degradation of HIF-1α. The compounds of the present invention also can be used to inhibit HIF-1α by interfering with the transcriptional activity of HIF-1α independent of its effect on the stability of the protein. Moreover, the mechanism by which the compounds of the present invention inhibit HIF-1α appears to be independent of the presence of oxygen or VHL. Therefore, the compounds of the present invention can be used to inhibit HIF-1α in hypoxic cells comprising VHL, or in normoxic or hypoxic cells lacking VHL or lacking normal VHL activity towards HIF-1α.

[0043] The present invention, therefore, provides a method of inhibiting HIF-1α in a cell. This method comprises administering to a cell comprising HIF-1α a compound of the present invention in an amount sufficient to inhibit HIF-1α. The method can be used with any type of cell, such as a mammalian cell, preferably, a human cell. A compound of the present invention can be administered to such cell *in vitro*, or *in vivo*, wherein the cell is in a host such as a mammal, preferably, a human.

[0044] A compound of the present invention is, desirably, administered to a cell comprising HIF-1α in an amount sufficient to inhibit HIF-1 α such that the activity of the HIF-1α in the cell is reduced by at least about 20%, preferably by at least about 30%, such as by at least about 40% (*e.g.*, by at least about 50%), by at least about 70% (*e.g.*, at least about 80%), or even by at least about 90%, or completely, as compared to the activity of HIF-1α in the cell in the absence of the compound of the present invention. Methods by which the inhibition of the HIF-1α protein activity can be measured are generally known in the art. For example, known methods can be used to measure and compare the level of HIF-1α produced in a cell, the half-life of HIF-1α in a cell, and/or the transactivation activity of HIF-1α in a cell, in the presence and absence of a compound of the present invention in order to evaluate the level of HIF-1α activity.

[0045] The above-described inventive method of inhibiting Hsp90, method of selectively inhibiting Hsp90 without substantially inhibiting Grp94, and method of inhibiting HIF-1α can be used for any purpose. Such uses will be apparent to those of ordinary skill in the art. For example, the inhibition or selective inhibition of Hsp90, and/or the inhibition of HIF-1α, can be used to investigate, for instance, intra- or inter-cellular molecular activity, such as the interactions between the proteins and compounds discussed herein and their relation to the diagnosis, treatment, and prevention of cancer.

[0046] Such methods are also advantageous for applications *in vivo*. For example, the activity of each of the Hsp90 and HIF-1α proteins has been linked to cancer cell proliferation, and the inhibition of such proteins is useful in the diagnosis, treatment, or prevention of cancer or tumors in a host. Also, the selective inhibition of Hsp90 without substantially inhibiting Grp94 is believed to provide other special advantages *in vivo*. Grp94 is localized to the endoplasmic reticulum and performs, or assists, certain protein processing functions. Without wishing to be bound by any particular theory, it is believed that the inhibition of Grp94 causes certain undesirable side effects without any substantial anti-cancer or anti-tumor effect. It is further believed that the use of geldanamycin or its known derivatives, which have approximately equal affinity for Hsp90 and Grp94, leads to unwanted side effects for these reasons. Thus, it is believed that the present method of selectively inhibiting Hsp90 can be used advantageously *in vivo* without substantially disrupting the function of Grp94, for example, in the treatment, diagnosis, or prevention of cancer.

[0047] In this regard, the present invention further provides a method of treating or preventing cancer in a host. The method comprises administering a compound of the present invention to a host in an amount sufficient to treat or prevent cancer in the host, whereupon the cancer of the host is treated or prevented. Desirably, the host includes cells comprising Hsp90 and Grp94, and the compound is administered in an amount sufficient to inhibit Hsp 90 without substantially inhibiting Grp94. Also desirably, the cells of the host further comprise ErbB2 proteins, and the compound is administered in an amount sufficient to deplete substantially ErbB2 proteins in the cells. The method of the present invention can also be used to treat or prevent cancer in a host that includes cells comprising HIF-1α, wherein a compound of the present invention is administered in an amount sufficient to inhibit HIF-1α. The method of the present invention is especially useful to treat or prevent cancer in a host, as described herein, wherein the cancer is a metastatic cancer, such as a renal cell carcinoma. Preferably, the host is a mammal, in particular a human.

[0048] The method of treating cancer using the compound of the present invention can be made more effective by administering one or more other anticancer compounds along with one or more other compounds of the present invention. These other anticancer compounds include, but are not limited to, all of the known anticancer compounds approved for marketing in the United States and those that will become approved in the future. See, for example, Table 1 and Table 2 of Boyd, *Current Therapy in Oncology*, Section I. Introduction to Cancer Therapy (J.E. Niederhuber, ed.), Chapter 2, by B.C. Decker, Inc., Philadelphia, 1993, pp. 11-22. More particularly, these other anticancer compounds include doxorubicin, bleomycin, vincristine, vinblastine, VP-16, VW-26,

cisplatin, carboplatin, procarbazine, and taxol for solid tumors in general; alkylating agents, such as BCNU, CCNU, methyl-CCNU and DTIC, for brain or kidney cancers; and antimetabolites such as 5-FU and methotrexate for colon cancer.

[0049] One skilled in the art will appreciate that suitable methods of administering compositions comprising the present inventive compound to an animal, such as a mammal, in particular a human, are available, and, although more than one route can be used to administer a particular compound, a particular route can provide a more immediate and more effective reaction than another route. Accordingly, the herein-described methods are exemplary and are in no way limiting.

[0050] The dose administered to an animal, such as a mammal, in particular a human, should be sufficient to prevent cancer, delay its onset, or slow (or stop) its progression. One skilled in the art will recognize that dosage will depend upon a variety of factors including the strength of the particular compound employed, as well as the age, species, condition, and body weight of the animal. The size of the dose will also be determined by the route, timing, and frequency of administration as well as the existence, nature, and extent of any adverse side-effects that might accompany the administration of a particular compound and the desired physiological effect.

[0051] Suitable doses and dosage regimens can be determined by conventional range-finding techniques known to those of ordinary skill in the art. Generally, treatment is initiated with smaller dosages, which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under the circumstances is reached. The present inventive method will typically involve the administration of about 0.1 to about 100 mg of one or more of the compounds described above per kg body weight.

EXAMPLES

[0052] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope. Three compounds of the present invention (i.e., Compounds A-E) were prepared as described below. These compounds were subsequently used in a hollow fiber assay (Example 1) to determine if the compounds produced sufficient *in vivo* activity so as to warrant further testing, competitive binding assays to determine Hsp90 (Example 2) and Grp94 (Example 3) binding affinities, and a western blot to measure the effect on ErbB2 protein levels in a host cell.

Preparation of Compound A

Preparation of Geldanamycinglycinate Hydrochloride

[0053] A mixture of geldanamycin (6 g, 10.7 mmol) (NCI Lot Nos. 3059-25-1 and 2047-72-1), 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-*p*-toluenesulfonate (5.3 g, 12.5 mmol) (Aldrich, Lot No. AF02307AZ), 4-dimethylaminopyridine (DMAP) (160 mg, 1.3 mmol) (Lancaster, Lot. No. P00472) and N-(tert-butoxycarbonyl) glycine (1.6 g, 9.13 mmol) (Aldrich, Lot No. MX08309EW) was stirred at room temperature overnight (ca. 18 hours). Two such reactions were carried out simultaneously. Both of these reaction mixtures were then filtered through the same celite pad. The resulting filtrate was washed with 5% $NaHCO_3$ (35 mL X 2) (J.T. Baker, Lot No. F24728) and water (35 mL X 3), dried using $MgSO_4$ (J.T. Baker, Lot No. F43145) and concentrated. The resulting crude product was triturated with water (100 mL X 3) and redissolved in ether (200 mL) (EM Science, Lot No. 32202). The ether solution was washed with water (70 mL X 4), dried using $MgSO_4$ and evaporated to give a solid (6 g), which was stirred in water (150 mL) at room temperature for 4 hours. The solid was collected and air-dried to give 3.8 g of product (Geldanamycin N-(tert-butoxycarbonyl) glycinate). This product was further purified by silica gel column chromatography using 5% MeOH/CH_2Cl_2 as eluent (2.4 g) to form an intermediate compound of Geldanamycin N-(tert-butoxycarbonyl) glycinate.

[0054] Trifluoroacetic acid (7.5 mL) (Aldrich, Lot No. PZ06421KZ) was added to a clear solution of the intermediate compound described above (1.4 g, 1.95 mmol) in dry methylene chloride (15 mL) (Burdick & Jackson, Lot No. BE512) at room temperature. The resulting clear red solution was stirred at room temperature for 30 minutes, evaporated and pumped (0.1 mmHg) to dryness. While still at room temperature, the residue was triturated with ether (30 mL) and filtered to give a yellow powder (1.2 g), which was subsequently dissolved in methylene chloride (30 mL). The resulting solution was then washed with 5% $NaHCO_3$ (10 mL X 2) and with brine (10 mL X 1), dried using $MgSO_4$ and filtered. The pH of the solution was then adjusted to ca. 3 by the addition of methanolic HCl. After adjusting the pH, the filtrate was evaporated and pumped (0.1 mmHg) to dryness. The remaining residue was dissolved in methylene chloride (2 mL) and subsequently diluted with dilute ethereal HCl (50 mL) at room temperature. This mixture was stirred for 30 minutes and filtered to give prepurified product.

[0055] To purify the product, the salt (1 g, 1.53 mmol) was redissolved in methylene chloride (50 mL). The solution was then washed with 1% $NaHCO_3$ (25 mL X 1) and water (25 mL X 2), dried using $MgSO_4$ and filtered. The filtrate was adjusted to pH 3 with dilute methanolic HCl to produce a red solution, which was subsequently evaporated and pumped (0.1 mmHg) to dryness. The residue was then dissolved in methylene

chloride (2 mL), seeded and diluted with dilute ethereal HCl (30 mL). The mixture was stirred at room temperature for 30 minutes and filtered to produce 4.8 g of purified Geldanamycinglycinate hydrochloride. The water-solubility of the purified product was greater than 3 mg/ml.

Preparation of Compound B

17-Demethoxy-17-allylamino geldanamycin-11-aminoacetate hydrochloride

[0056] A mixture of geldanamycinglycinate hydrochloride (900 mg, 1.38 mol) (NCI Supplies, Lot No. ML-04-02) and allylamine (1.04 mL, 13.8 mmol) (Acros Organics, Lot No. 81223/1) in methylene chloride (36 mL) (J.T. Baker, Lot No. J51613) was stirred at room temperature for 18 hours. The reaction mixture was evaporated in an aspirator to give a purple gummy residue (1.0 g). A second run (800 mg) was carried out in a similar manner and gave 910 mg of residue. The residues were combined and subsequently purified by silica gel chromatography (36 g) eluting with methylene chloride (100 mL) followed by methylene chloride-methanol (195:5, 200 mL; 97:3, 200 mL; 193:7, 200 mL; 96:4, 200 mL; 95:5, 200 mL, 94:6, 200 mL). The product-containing fractions were combined and concentrated in an aspirator to dryness. The residue (1 g) was dissolved in methylene chloride (10 mL) and the solution was filtered through a celite pad (Celite Corp., Lot No. 96316). To the stirred filtrate was added 1 mL of cold 1.2 M hydrogen chloride-ethanol solution, which was prepared by passing hydrogen chloride gas (Matheson, Lot No. T410240) into ethanol, followed by diethyl ether (100 mL) (J.T. Baker, Lot No. K42582). The resulting mixture was refrigerated for 1 hour and filtered. The solid was washed with diethyl ether (3 X 20 mL) and dried at 64 °C/0.1 mmHg for 5 hours to give 830 mg (47%) of target compound as a purple solid. The water solubility of the purified product was greater than 5 mg/mL.

Preparation of Compound C

17-Demethoxy-17-allylamino geldanamycin-11-[3-(dimethylamino)-propionate] hydrochloride

[0057] A mixture of methyl 3-(dimethylamino) propionate (5 g, 38.1 mmol) (Aldrich, Lot No. 10016AN) in 3 N hydrochloric acid (30 mL) (Chempure, Lot No. M152KLTS) was heated on a steam bath for 1 hour with stirring and then concentrated in an aspirator to a volume of about 8 mL. The resulting mixture was cooled to room temperature and quenched with acetone (30 mL) (Mallinckrodt, Lot No. 2440KTML). The solid was collected by filtration, washed with acetone (2 X 10 mL) and air-dried to give 4.7 g of 3-(dimethylamino) propionic acid hydrochloride as a white crystalline solid (80%), mp 188-192 °C.

[0058] A suspension of 3-(dimethylamino) propionic acid hydrochloride (4.7 g, 30.6 mmol) in thionyl chloride (20 mL) (Fluka, Lot No. 340130/11194) was heated to reflux in a steam bath for 30 minutes to form a clear yellow solution. The excess thionyl chloride was removed by distillation to give a light brown residue. The residue was then triturated with anhydrous ether (3 X 10 mL) (Fisher Scientific, Lot No. 96732415) under nitrogen and dried in a nitrogen stream to give 5.0 g of 3-(dimethylamino) propionic acid chloride as a light brown solid. This material was taken on directly to the next step without further purification.

[0059] A solution of 17-demethoxy-17-allylamino geldanamycin (1 g, 1.7 mmol) (Ash Stevens Inc., Lot No. BK-10-303) in methylene chloride (20 mL) was added to 3-(dimethylamino) propionic acid chloride (0.4 g) with stirring at room temperature. The reaction mixture was stirred for 1 hour and concentrated in an aspirator to give a purple gummy residue, which was chromatographed over silica gel (35 g). The column was eluted with methylene chloride (100 mL) followed by methylene chloride-methanol (97:3, 200 mL; 96:4, 100 mL; 95:5, 100 mL; 94:6, 100 mL; 93:7, 100 mL; 92:8, 100 mL). The product-containing fractions were combined and concentrated in an aspirator to give a residue which was rechromatographed over silica gel (27 g) using methylene chloride-methanol (97:3, 100 mL; 193:7, 200 mL; 96:4, 200 mL; 95:5, 100 mL; 94:6, 200 mL; 93:7, 200 mL) as eluant. The product-containing fractions were combined and concentrated in an aspirator to give a residue, which was dissolved in a mixture of methylene chloride (3 mL) and diethyl either (5 mL). To the stirred solution, cooled in an ice bath, was added cold 1.2 M hydrogen chloride-ethanol solution (0.8 mL) followed by diethyl ether (15 mL). The mixture was refrigerated for 1 hour and filtered. The solid was washed with diethyl ether (2 X 10 mL) and air-dried to give 600 mg of target compound. A second run (1 g) was carried out in the same manner and gave 580 mg of target compound. The combined product from both runs (1.18 g) was dissolved in methylene chloride (10 mL) and filtered through a celite pad (Celite Corp., Lot No., 96316). The filtrate was diluted with diethyl ether (60 mL) with stirring, then refrigerated for 1 hour. The solid was collected by filtration, washed with diethyl ether (3 X 8 mL), and dried at 64 °C/0.1 mmHg for 4 hours to give 960 mg (39%) of pure target compound as a purple solid. The water solubility of the purified product was greater than 3 mg/mL.

Preparation of Compound D

Preparation of 11-(4-aminobutyrate)-geldanamycin hydrochloride

[0060] 4-aminobutyric acid (44.0 g, 0.43 mol) was added to a solution of sodium carbonate (22.0 g, 0.21 mol) and water (160 ml). The resulting clear solution was diluted with dioxane (160 ml) and di-tert-butyldicarbonate (110 g, 0.50 mol) was added to the

dilution. The mixture was stirred at room temperature overnight (approximately 18 hours), after which the solvent was evaporated. Water (660 ml) was added to the residue and the mixture was extracted three times with ethyl acetate (400 ml aliquots). The pH of the aqueous layer was adjusted from a pH of 9 to pH of 3 by the addition of solid sodium bisulfate monohydrate (59.0 g, 0.43 mol), and the pH-adjusted solution was extracted three times with ether (450 ml aliquots). The combined ether extract was washed with water (200 ml), dried using $MgSO_4$, and concentrated. The residue (87.9 g) was dissolved in ether (100 ml). The solution was diluted with petroleum ether (50 ml), seeded with product from a previous preparation and cooled in an ice-water bath. After the product crystallized, more petroleum ether (250 ml) was added and the mixture was stirred at 0 ^{0}C for 1 hour. The white solid was collected by filtration to give 71.9 g of 4-(tert-butoxycarbonyl)-aminobutyric acid (mp 50-52 ^{o}C).

[0061] A mixture of geldanamycin (10.0 g, 17.8 mmol), 1,3-diisopropylcarbodiimide (8.0 g, 63.4 mmol), 4-dimethylamino-pyridine (DMAP) (0.8 g, 6.5 mmol), and 4-(tert-butoxycarbonyl) aminobutyric acid (9.4 g, 46.2 mmol) in dry methylene chloride (300 ml) was stirred at room temperature for 24 hours. Two such reactions were carried out simultaneously. Both of the reaction mixtures were filtered through the same celite pad. The filtrate was washed twice with aqueous 5% $NaHCO_3$ (100 ml aliquots) and with water (100 ml), then dried using $MgSO_4$ and concentrated. The residue was dissolved in the minimum volume of refluxing ethanol (1 L). The solution was cooled to room temperature and stirred for 3 hours. The yellowish orange powder was collected by filtration, washed twice with ethanol (30 ml aliquots), three times with arid petroleum ether (50 ml aliquots), and air-dried to give 13.6 g of pure 11-(4-(tert-butoxycarbonyl) aminobutyrate-geldanamycin (mp 235 ^{0}C). Additional geldanamycin (70.0 g) was processed in this manner to give an additional 43.7 g of the intermediate product.

[0062] The unreacted geldanamycin was recovered according to the following process. The mother liquor recovered from the production of the 11-(4-(tert-butoxycarbonyl) aminobutyrate-geldanamycin was concentrated to a small volume (100 ml) and the mixture was filtered. The solid was washed twice with ethanol (10 ml aliquots), three times with petroleum ether (20 ml aliquots), and air-dried to yield unreacted geldanamycin (24.4 g) contaminated with 11-(4-(tert-butoxycarbonyl) aminobutyrate-geldanamycin. All of the recovered geldanamycin was recycled to give additional intermediate (13.9 g, mp 235 ^{o}C), with acceptable elemental analysis.

[0063] Thus, a total of 90.0 g of fresh geldanamycin and 24.4 g of recovered material was processed to give 71.2 g of pure 11-(4-(tert-butoxycarbonyl) aminobutyrate-geldanamycin.

[0064] Trifluoroacetic acid (50 ml) was added to a clear solution of 11-(4-(tert-butoxycarbonyl) aminobutyrate-geldanamycin (10.0 g, 13.4 mmol) in dry methylene chloride (150 ml) at room temperature. The red solution was stirred at room temperature for 30 minutes, then evaporated and pumped (0.3 mmHg) to dryness.

[0065] An identical scale reaction was carried out simultaneously. Each residue was triturated with ether (60 ml) at room temperature, then diluted with petroleum ether (120 ml). Each mixture was stirred at room temperature for 30 mm and filtered through the same filter paper. The combined solid was dissolved in methylene chloride (300 ml). The solution was washed twice with 5% $NaHCO_3$ (aq.) (100 ml aliquots), once with water (100 ml), dried with $MgSO_4$, and filtered through a celite pad. The filtrate was acidified with methanolic HCl. The solution was evaporated and pumped dry to give 20.1 g of crude 11-(4-aminobutyrate)-geldanamycin hydrochloride.

[0066] In the same manner, additional 11-(4-(tert-butoxycarbonyl) aminobutyrate-geldanamycin (50.0 g total in several batches) was deprotected and converted to 50.3 g of crude 11-(4-aminobutyrate)-geldanamycin hydrochloride. The combined crude product (70.4 g) was dissolved in methylene chloride (500 ml) and the solution was filtered through a celite pad. The filtrate was concentrated to a volume of about 200 ml, then diluted with ether (600 ml) and stirred at room temperature for 1 hour and filtered. The orange, powdery solid (57.2 g) was suspended in hot ethanol (100 ml). The partial solution was diluted slowly with petroleum ether (800 ml). The mixture was stirred at room temperature for 1 hour. The yellow powder was collected by filtration and dried at 25 °C and at 0.1 mmHg for 10 hours to give 54.2 g of pure 11-(4-aminobutyrate)-geldanamycin hydrochloride. The water solubility of the purified product was about 3 mg/ml.

Preparation of Compound E

Preparation of 17-Demethoxy-17-allylamino geldanamycin-11-[4-(dimethylamino)-butyrate]

[0067] A suspension of 4-(dimethylamino) butyric acid hydrochloride (3 g, 17.9 mmol) (Aldrich, Lot No. 09128AR) in thionyl chloride (20 mL) (Fluka, Lot No. 340130/1 1194) was heated at reflux on a stream bath for 30 min to form a clear yellow solution. The excess thionyl chloride was removed by distillation to give a light brown residue. This material was triturated with anhydrous ether (10 mL X 3) (Fisher Scientific, Lot No. 967324-15) under a nitrogen atmosphere and dried in a nitrogen stream to give 3.3 g of acid chloride as a light brown solid.

[0068] To a solution of 17-demethoxy-17-allylamino geldanamycin (500 mg, 0.85 mmol) (Ash Stevens Inc., Lot No. BK-10-303) in methylene chloride (8 mL) (J.T. Baker, Lot No. K33619) was added pyridine (0.1 mL) (J.T. Baker, Lot No. E13618) and thionyl

chloride (300 mg). The reaction mixture was stirred at room temperature for 18 hours and then partitioned between methylene chloride (50 mL) and a 0.2 M sodium hydroxide solution (30 mL) (Aldrich, Lot No. M152KPCX). The organic phase was separated, washed successively with water (20 mL X 2) and brine (20 mL), dried over anhydrous $MgSO_4$ (EM Science, Lot No. 33146334), and filtered. The residue was purified by silica gel chromatography (20 g) eluting with methylene chloride (50 mL) followed by methylene chloride-methanol (97:3, 200 mL; 96:4, 200 mL); 95:5, 100 mL; 94:6, 100 mL; 93:7, 200 mL; 92:8, 200 mL) (Fisher Scientific, Lot No. 966097). The fractions containing product were combined and concentrated in an aspirator to dryness. The solution was filtered through a celite pad, and the filtrate was diluted with hexanes (30 mL) (Fisher Scientific, Lot No. 963814) and refrigerated for 3 hours. The solid was then collected by filtration and washed with hexanes (10 mL X 2), then air-dried to give 260 mg of target compound as a purple crystalline solid. In a similar manner, additional target compound was prepared in two batches and added to the first batch. The combined solids were then dried at 64 °C/0.1 mmHg for 5 hours to give 760 mg (32%) of 17-Demethoxy-17-allylamino geldanamycin-11-[4-(dimethylamino)-butyrate] as a purple crystalline solid.

The HFA and MTT Assays

[0069] Compounds A-E were tested in a hollow fiber assay for *in vivo* activity. The compounds were tested against a standard panel of 12 human tumor cell lines including NCI-H23, NCI-H522, MDA-MB-231, MDA-MB-435, SW-620, COLO 205, LOX IMVI, UACC-62, OVCAR-3, OVCAR-5, U251 and SF-295. The cell lines were cultivated in RPMI-1640 containing 10% FBS and 2 mM glutamine. On the day preceding hollow fiber preparation, the cells were given a supplementation of fresh medium to maintain log phase growth. For fiber preparation, the cells were harvested by standard trypsinization technique and resuspended at the desired cell density. The cell suspensions were flushed into 1 mm polyvinylidene fluoride (PVDF) hollow fibers (MW exclusion of 500,000 Da) and subsequently heat-sealed at 2 cm intervals. The samples generated from these seals were placed into tissue culture medium and incubated at 37 °C in 5% CO_2 for 24-48 hours prior to implantation. A total of 3 different tumor cell lines were prepared for each experiment so that each mouse received 3 intraperitoneal implants (1 of each tumor cell line) and 3 subcutaneous implants (1 of each tumor cell line). A total of 4 experiments were conducted on each mouse (3 cell lines/experiment X 4 experiments = 12 cell lines).

[0070] On the day of implantation, samples of each tumor cell line were quantitated for viable cell mass by a stable endpoint MTT assay so that the time 0 cell mass was known. In the MTT assay, the fibers were incubated in 6-well plates (1-6 fibers/well) with 3 mL of pre-warmed (37 °C) complete medium containing MTT (1 mg/mL) (Sigma) for 4

hours at 37 °C in 5% CO_2. The MTT solution was aspirated and the fibers were washed with 2 mL of saline containing 2.5% protamine sulfate overnight at 4 °C. A second wash was performed with 2 mL of saline containing 2.5% protamine sulfate, and the fibers were maintained at 4 °C for a minimum of 2 hours. The fibers were then removed from the protamine sulfate, individually wiped, placed in a 24-well plate (1 fiber/well), cut in half and dried overnight in a biosafety hood. To extract the formazan, DMSO was added (0.25 mL) to each well and the plates were rotated for 4 hours at room temperature. The extracted samples were transferred to 96-well plates and the OD was determined at 540 nm. From these spectrophotomeric measurements, the cytostatic and cytocidal capacities of the test compound were assessed.

[0071] Following implantation of the hollow fibers, mice were treated with Compounds A-E on day 3 or 4 following fiber implantation and continuing once daily for a total of 4 doses. The compounds were assessed by intraperitoneal injection at 2 dose levels with 3 mice/dose/experiment. Vehicle controls consisted of 6 mice receiving the compound diluent only. The fibers were collected from the mice on the day following the fourth compound treatment and subjected to the stable endpoint MTT assay. The optical density of each sample was determined spectrophotometrically at 540 nm and the mean of each treatment group was calculated. The percent net cell growth in each treatment group was determined and compared to the percent net cell growth in the vehicle treated controls.

[0072] Compounds were scored on the basis of several hollow fiber assay criteria, including (1) a reduction in net cell growth of 50% or greater in 10 of the 48 possible test combinations; (2) a reduction in net cell growth of 50% or greater in a minimum of 4 of the 24 distant site combinations (SC score); and/or (3) cell kill of 1 or more cell lines in either implant site (reduction in the viable cell mass below the level present at the start of the experiment).

[0073] To simplify evaluation, a point system was adopted which allows rapid viewing of the activity of a given compound. For this, a value of 2 was assigned for each compound dose, which results in a 50% or greater reduction in viable, cell mass. The intraperitoneal (IP) and subcutaneous (SC) samples were scored separately so that criteria (1) and (2) could be evaluated independently.

Example 1

[0074] This example demonstrates the *in vivo* activity of compounds of the present invention.

[0075] A hollow fiber assay was conducted with Compounds A-E, as described above. Compounds A-E were mixed with saline and tween 80 (0.05%) to obtain the dosage levels

indicated in Table 1. Optimal dosage levels were determined from previous toxicity studies and therefore the dosages used represent the upper range that can be administered to a host without inducing toxic effects. The compounds were administered once daily for four days via intraperitoneal injection. After four days, the hollow fibers were removed from the mice and subjected to the MTT assay as described above. The percent cell growth inhibition was calculated [(1-(% treated net growth/% control net growth))*100] and an HFA score was assigned to Compounds A-E on the basis of this calculation, as indicated in Table 1.

Table 1

Compound	Route of Administration	Dose/Units (mg/kg/dose)	IP Score	SC Score	Total HFA Score
A	IP	50	16	4	20
B	IP	100	20	12	32
C	IP	100	16	2	18
D	IP	50	8	8	16
E	IP	100	8	8	16

[0076] Based on the results indicated above, Compounds A-E of the present invention exhibited significant *in vivo* anti-cancer activity.

Example 2

[0077] This example demonstrates the relative affinity of GA and a compound of the present invention (11-(4-aminobutyrate)-geldanamycin hydrochloride) for Hsp90.

[0078] Hsp90 binding affinity was analyzed through competitive binding between the soluble test drug (GA or 11-(4-aminobutyrate)-geldanamycin hydrochloride) and immobilized GA resin. SKBR3 cells (purchased from American Type Culture Collection, Manassas, VA) were cultured in DMEM medium supplemented with 10% FCS, 2 mM glutamine, 1 mM Hepes, and 100 U penicillin and streptomycin, for 24-48 hours. The cells were washed once with cold phosphate-buffered saline (pH=7.0), and lysed by scraping in TMNSV (50 mM Tris-HCl (pH=7.5), 20 mM Na2MoO4, 0.09 wt.% NP-40, 150 mM NaCl, and 1 mM sodium othovanadate, supplemented with proteinase inhibitors (CompleteÔ by Roche)). Cell lysates were clarified by centrifugation at 14,000 rpm (4 °C) for 15 minutes.

[0079] Solutions of GA or 11-(4-aminobutyrate)-geldanamycin hydrochloride at concentrations from 0.002 μM to 8 μM were incubated on ice with cell lysates for 30 minutes. After incubation, 100 μl of GA resin (50 wt.% suspension) was added to the

lysate/drug mixture and the volume was adjusted to 500 µl with lysis buffer. The samples were rotated at 4 °C for two hours. The GA resin was collected, washed four times in lysis buffer, and combined with 100 µl of 1x SDS sample buffer. The samples were separated by 8% SDS-PAGE gel electrophoresis and transferred to nitrocellulose paper by Western Blotting. The blots were probed for Hsp90 and band densities were obtained by densitometry using Adobe Photoshop and NIH image software.

[0080] The results indicated that 11-(4-aminobutyrate)-geldanamycin hydrochloride competed with immobilized GA for Hsp90 binding with an approximate IC50 of 1 µM, while GA competed with immobilized GA with an IC50 of about 0.3 µM. These results demonstrate that the compound of the present invention effectively binds Hsp90, having only about a 3-fold weaker affinity for Hsp90 than GA.

Example 3

[0081] This example demonstrates the relative affinity of GA and a compound of the present invention (11-(4-aminobutyrate)-geldanamycin hydrochloride) for Grp94.

[0082] Grp94 binding affinity was determined through competitive binding between the soluble test drug (GA or 11-(4-aminobutyrate)-geldanamycin hydrochloride) and [3H]-N-ethylcarboxamidoadenosine (NECA). GA and NECA are known to share the same binding site on Grp94. The assay solutions contained 5 mg of purified Grp94, 20 nM of NECA, 50 mM Tris buffer (pH at 7.5), and concentrations of GA or 11-(4-aminobutyrate)-geldanamycin hydrochloride ranging from 0.03 µM to 100 µM. The assay solutions were incubated on ice for 1 hour. Grp94 was then collected on polyethyleneimine-treated glass filters and washed twice with 4 ml of ice-cold 50 mM Tris (pH at 7.5). The washed Grp94 was dried and counted in a scintillation counter to determine the amount of NECA-bound Grp94.

[0083] The results indicated that GA competes with [3H]-NECA for Grp94 binding with an approximate IC50 of 1 µM. By comparison, 11-(4-aminobutyrate)-geldanamycin hydrochloride competes with [3H]-NECA for Grp94 binding with an IC50 of about 90 µM. These results demonstrate that the compound of the present invention selectively binds to Hsp90 over Grp94, having about a 90-fold weaker affinity for Grp94 than GA.

Example 4

[0084] This example demonstrates the use of a compound of the present invention (11-(4-aminobutyrate)-geldanamycin hydrochloride) to deplete the concentration of ErbB2 in a host cell.

[0085] SRKB3 cells, as used in Example 2, were incubated with increasing concentrations of GA or 11-(4-aminobutyrate)-geldanamycin hydrochloride. Cells were

lysed and ErbB2 content of the total lyses were determined by SDS-PAGE gel electrophoresis followed by Western Blotting for ErbB2. For purposes of comparison, the same procedure was carried out using GA.

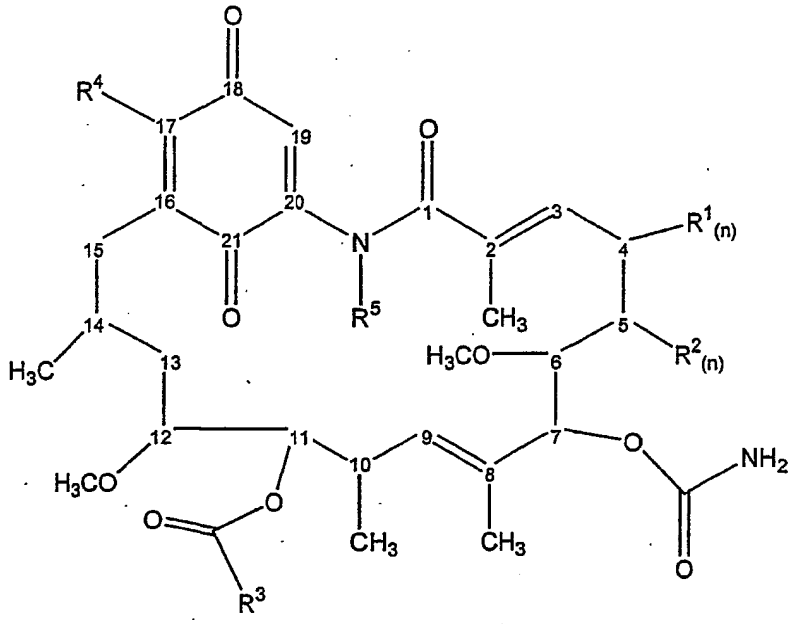
[0086] The results indicated that 50% of ErbB2 was depleted from cells incubated with the 3.5 μM 11-(4-aminobutyrate)-geldanamycin hydrochloride solution, and ErbB2 depletion was complete using the 10 μM solution. By comparison, ErbB2 depletion was complete using a 1 μM solution of GA. These results demonstrate that the compound of the present invention can be used to reduce effectively or eliminate the production of ErbB2 in a host, while only slightly less effective than natural GA.

[0087] All of the references cited herein, including patents, patent applications, and publications, are hereby incorporated in their entireties by reference.

[0088] While this invention has been described with an emphasis upon preferred embodiments, variations of the preferred embodiments can be used, and it is intended that the invention can be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the claims.

We claim:

1. A compound of formula (I)



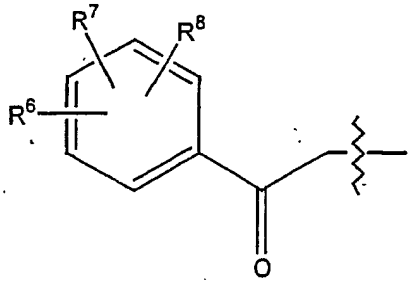
(I)

wherein n=0 or 1 and wherein, when n=1, each of R^1 and R^2 is hydrogen, and, when n=0, a double-bond exists between 4 and 5;

R^3 is a C_1-C_8 aminoalkyl or a C_1-C_8 iminoalkyl;

R^4 is hydrogen, a methoxy, a C_1-C_8 alkylamino, a C_1-C_8 dialkylamino, a C_1-C_8 N,N'-dialkylaminoalkylamino, or an allylamino; and

R^5 is hydrogen or a group of the formula



wherein each of R^6, R^7, and R^8 is independently selected from the group consisting of hydrogen, a halo, an azido, a nitro, a C_1-C_8 alkyl, a C_1-C_8 alkoxy, an aryl, a cyano, and

an $NR^{10}R^{11}R^{12}$, wherein each of R^{10}, R^{11}, and R^{12} is independently selected from the group consisting of hydrogen and a C_1-C_3 alkyl; and salts thereof and prodrugs thereof.

2. The compound of claim 1, wherein R^3 is a C_1-C_8 aminoalkyl or a C_1-C_8 iminoalkyl that is substituted with one or more C_1-C_4 alkyl groups, which can be the same or different.

3. The compound of claim 2, wherein R^3 is a C_1-C_8 N-methylaminoalkyl or a C_1-C_8 N,N'-dimethylaminoalkyl.

4. The compound of claim 3, wherein R^3 is N,N'-dimethylaminopropyl.

5. The compound of claim 3, wherein R^3 is N-N'-dimethylaminoethyl.

6. The compound of any of claims 1-3, wherein R^3 is selected from the group consisting of an aminomethyl, an aminoethyl, an aminopropyl, and an aminobutyl.

7. The compound of claim 6, wherein R^3 is an aminomethyl.

8. The compound of claim 6, wherein R^3 is an aminopropyl.

9. The compound of any of claims 1-8, wherein n =0.

10. The compound of any of claims 1-9, wherein, when R^4 is a methoxy, a C_1-C_8 alkylamino, a C_1-C_8 dialkylamino, a C_1-C_8 N,N'-dialkylaminoalkylamino, or an allylamino, R^4 is substituted with one or more substituents, which can be the same or different, selected from the group consisting of a C_1-C_4 alkyl, a C_1-C_8 aryl, a halo, a cyano, a nitro, an azido, an amido, and an amino.

11. The compound of any of claims 1-9, wherein R^4 is methoxy.

12. The compound of any of claims 1-9, wherein R^4 is an allylamino.

13. The compound of any of claims 1-12, wherein R^5 is hydrogen.

14. The compound of any of claims 1-13, wherein the compound has a binding affinity for Hsp90 that is at least about $1/10^{th}$ or more of the binding affinity of

geldanamycin for Hsp90.

15. The compound of any of claims 1-14, wherein the compound has a binding affinity for Grp94 that is about $1/20^{th}$ or less than the binding affinity of geldanamycin for Grp94.

16. The compound of any of claims 1-15, wherein the compound inhibits HIF-1α.

17. The compound of any of claims 1-16, wherein the compound has improved water-solubility as compared to geldanamycin.

18. A pharmaceutical composition comprising a compound of any of claims 1-17 and a pharmaceutically acceptable carrier.

19. The pharmaceutical composition of claim 18, wherein the compound is present in an amount sufficient to inhibit Hsp90 without substantially inhibiting Grp94.

20. The pharmaceutical composition of claim 18 or 19, wherein the compound is present in an amount sufficient to inhibit HIF-1α.

21. A method of selectively inhibiting Hsp90 without substantially inhibiting Grp94 in a cell comprising administering to a cell comprising Hsp90 and Grp94 a compound of any of claims 1-17 in an amount sufficient to inhibit Hsp90 without substantially inhibiting Grp94, whereupon Hsp90 is selectively inhibited.

22. A method of inhibiting HIF-1α in a cell comprising administering to a cell comprising HIF-1α a compound of any of claims 1-17 in an amount sufficient to inhibit HIF-1α.

23. The method of claim 21 or 22, wherein the cell is in a host.

24. A method of treating or preventing cancer in a host, which method comprises administering a compound of any of claims 1-17 to a host in an amount sufficient to treat or prevent cancer in the host, whereupon the cancer of the host is treated or prevented.

25. The method of claim 24, wherein the host includes cells comprising Hsp90 and Grp94, and the compound is administered in an amount sufficient to inhibit Hsp90 without substantially inhibiting Grp94.

26. The method of claim 25, wherein the cells further comprise ErbB2 proteins, and the compound is administered in an amount sufficient to deplete substantially ErbB2 proteins in the cells.

27. The method of any of claims 23-25, wherein the host is a mammal.

28. The method of claim 27, wherein the mammal is a human.

INTERNATIONAL SEARCH REPORT

Inter il Application No
PCT/US 01/44172

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D225/06 A61K31/395 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, CHEM ABS Data, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 45805 A (HOSE CURTIS D ;US HEALTH (US); MONKS ANNE (US); WEBB CRAIG P (US);) 10 August 2000 (2000-08-10) figures 18,19,22,23 claims 1,12,13,16,17 ---	1-28
X	WO 95 01342 A (PFIZER ;GALLASCHUN RANDALL JAMES (US); MOYER MIKEL PAUL (US); SCHN) 12 January 1995 (1995-01-12) claims 10-19 examples 62,63 --- -/--	1-28

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

'A' document defining the general state of the art which is not considered to be of particular relevance

'E' earlier document but published on or after the international filing date

'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

'O' document referring to an oral disclosure, use, exhibition or other means

'P' document published prior to the international filing date but later than the priority date claimed

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

'&' document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 March 2002	03/04/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Seitner, I

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INTERNATIONAL SEARCH REPORT

Interr Application No
PCT/US 01/44172

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	SCHNUR, R. C. ET AL: "erbB-2 oncogene inhibition by geldanamycin derivatives: synthesis, mechanism of action, and structure-activity relationships" J. MED. CHEM. (1995), 38(19), 3813-20 , XP002193280 examples 3A-3G; table 1 -----	1,24

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Claims 21 and 22 are directed to a method of treatment of the human/animal body in as far as the cell is in a human/animal body. For those parts of claims 21 and 22 the search has been carried out and based on the alleged effects of the compound.
Moreover, claims 23-28 are also directed to a method of treatment of the human/animal body and the search has therefore been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.2

Claims Nos.: 1-28 (all partially)

The scope of the claims 1-28, in as far as the expression "prodrugs" (see claim 1, page 26) is concerned, is so unclear (Article 6 PCT) that a meaningful International Search is impossible with regard to this expression.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern Application No
PCT/US 01/44172

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0045805 A	10-08-2000	AU 2986800 A	25-08-2000
		WO 0045805 A2	10-08-2000
WO 9501342 A	12-01-1995	CA 2166320 A1	12-01-1995
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		FI 943100 A	30-12-1994
		WO 9501342 A1	12-01-1995
		JP 2794342 B2	03-09-1998
		JP 8506356 T	09-07-1996